REMARKS

In the claim listing above, claims 112, 114, 117, 120, 123, 125, 127, 130, 133, 135, 138, 139, 141, 143, 145, 146 and 148 have been amended. Claims 121-122 and 131-132 have been canceled. No claims have been added. Accordingly, as amended above, claims 112-120, 123-130 and 133-148 are presented for further examination in this application.

Changes to the Claims

As just indicated, claims 112, 114, 117, 120, 123, 125, 127, 130, 133, 135, 138, 139, 141, 143, 145, 146 and 148 have been amended in the claim listing above.

Claims 112, 123, 133, 143 and 146 are independent. Each has been amended to define more clearly the nature of the copy which is produced by Applicants' claimed processes. For example, claims 112, 123, 143 and 146 now recite *DNA molecule of interest* instead of "nucleic acid of interest" (claims 112 and 123) or "specific nucleic acid" (claims 143 and 146). Claim 133 now recites *more than one complementary copy of an RNA molecule of interest* instead of "RNA of interest."

Further changes to the independent claims have been made. Step (c) in claim 112 has been amended to recite *carrying out nucleic acid synthesis to extend a primer bound to said DNA molecule of interest and produce a polynucleotide comprising an RNA/DNA hybrid, thereby generating a substrate for RNase H.* The word *extended* has also been inserted in front of "primer" in step (d) in claim 112.

Similar changes have been made to independent claim 123. Step (c) in claim 123 now recites *producing at least one copy of said DNA molecule of interest by using said nucleic acid producing catalyst (iii) and said DNA*

molecules of interest as templates to extend said copolymer primer. The final step (d) in claim 123 now recites *removing said RNA segment of said extended copolymer primer from said template by digesting with RNase H to bind another copolymer primer to said template and initiate synthesis, thereby multiply initiating polynucleotide or oligonucleotide synthesis.*

In addition to the substitution of *DNA molecule* for "specific nucleic acid," claim 143 has been amended in step (c). That step now calls for *allowing said mixture to react under isostatic conditions of temperature, buffer and ionic strength, thereby producing at least one copy of said DNA molecule by extension of said primer*.

Similarly, in claim 146, two steps have been amended. Step (b)(iii) calls for *an effective amount of a reverse transcriptase having RNase activity*. Step (c) now recites *allowing said mixture to react under isostatic conditions of temperature, buffer and ionic strength, thereby producing at least one copy of said DNA molecule by extension of said primer*.

To conform with the *DNA molecule* language in claim 112, claim 114 has been amended to recite the same term. In claim 117, "RNA polymerase" has been removed from the list of recited members for nucleic acid producing catalysts (iii). Claim 120 now recites *labeled nucleic acid precursors*.

To conform with the *DNA molecule* language in claim 123, the same term has been substituted in claim 125. As in the case of claim 117, the term "RNA polymerase" has been deleted from claim 127. As in the case of claim 120, *labeled nucleic acid precursors* are now recited in claim 130. A minor typographical error has also been corrected in claim 130.

Claim 145 has been amended to recite *DNA molecule* in order to conform with the language in independent claim 143 from which it depends. A similar change has been made to claim 148 which depends from independent claim 146.

It is believed that the foregoing amendments are supported by Applicants' original disclosure and, therefore, constitute subject matter to which they are duly entitled to claim.

Entry of the above amendments and new claim listing is respectfully requested.

Priority and Withdrawn Objections and Rejections

Applicants acknowledge with appreciation the indication in the Office Communication that their claim for priority (01/13/94) has been granted. They also appreciate the indication that the objections to the abstract and specification have been mooted based upon amendments to the specification.

Commonality of Ownership

Applicants assert that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made.

The First Rejection Under 35 U.S.C. §103

Claims 112-121, 123-130, 133-143, 145-146, 148 are rejected under 35 U.S.C. §103(a) as being unpatentable over Schuster et al. (US Patent 5,169,766 December 8, 1992) in view of Scheele (US Patent 5162209 November 10, 1992). According to the Office Communication (pages 3-7):

> With regard to Claim 112 and 123, Schuster et al. teaches a method of amplifying a nucleic acid molecule. Schuster et al. teaches providing a DNA target (Figure 3 ssDNA analyte). Schuster et al. teaches mixing the target with nucleoside triphosphates (nucleic acid precursors) (Column 7, lines 60-65). Schuster et al. teaches the proto-primer used can be a RNA sequence (Column 11, lines 47-51). Schuster et al. teaches the use of RNase H to remove RNA from the cDNA (Column 8, lines 7-10).
>
> Schuster et al. teaches making RNA:DNA hybrid (Column 9, lines 32-33). Schuster et al. teaches an mRNA promoter (primer) which is used

to extend and make 5sRNA (Figure 3).

Schuster et al. teaches another primer (DNA) is annealed to the ssRNA and cDNA is copied (Figure 3).

Schuster et al. teaches the ssRNA (which is the extended promoter-primer) is destroyed by RNase H (Figure 3). Schuster et al. teaches that this method cyclical and therefore can produce multiple copies (Column 4 lies 50-54). Therefore after the RNA is destroyed another primer can anneal to the nucleic acid sample.

With regard to Claim 133, Schuster et al. teaches a method of amplification in which the desired nucleic acid molecules can be RNA (Column 9, lines 52-53). Schuster et al. teaches a method of amplification in which a starting ssRNA analyte is primed in conditions for replication, a double stranded DNA template is produced, and RNase H is used to remove the SNA primers segment to allow the next priming event to occur (Figure 2).

With regard to Claims 113, 124, and 134, Schuster et al. teaches the use of primers which are chemically modified by blocking the 3' terminus using a 3' terminal nucleotide lacking a 3' hydroxyl group (modified nucleotides) (Column 11 lines 60-64).

With regard to Claims 114-115, 125-126, 135-136, and 142, Schuster et al. teaches a promoter primer which has at least 1 noncomplementary nucleotides (Figure 1 2nd step).

With regard to Claims 116, and 137, Schuster et al. teaches that the modified proto-primer can be RNA; therefore it is inherent that an RNA strand would be composed of deoxyribonucleotides (Column 11, lines 47-60).

With regard to Claims 117-119, 127-130, and 138-140, Schuster et al. teaches DNA polymerase (nucleic acid catalyst) include Taq polymerase, Klenow polymerase, E. coli polymerase, and 17 DNA polymerase (Column 7, lines 14-20).

With regard to Claims 120 and 141, Schuster et al. teaches mixing the target with nucleoside triphosphates (nucleic acid precursors) (Figure 1 Column 7, lines 60-65).

With regard to Claims 121, Schuster et al. teaches the use of primers which are chemically modified by blocking the 3' terminus using a 3' terminal nucleotide lacking a 3' hydroxyl group (Column 11 lines 60-64).

With regard to Claim 143 and 146, Schuster et al. teaches a method of amplifying a nucleic acid molecule. Schuster et al. teaches providing a DNA target (Figure 3 ssDNA analyte). Schuster et al. teaches mixing the target with nucleoside triphosphates (nucleic acid precursors) (Column 7, lines 60-65). Schuster et al. teaches the proto-primer used can be a RNA sequence (Column 11, lines 47-51). Schuster et al.

teaches the addition of a polymerase (Column 7, lines 14-20). Schuster et al. teaches the use of RNase H to remove RNA from the cDNA (Column 8, lines 7-10, Figure 3).

Schuster et al. teaches producing a copy of a specific nucleic acid (Figure 3) and removing ribonucleotides using RNase H (Figure 3). Schuster et al. teaches an mRNA promoter (primer) which is used to extend and make ssRNA (Figure 3). Schuster et al. teaches the ssRNA (which is the extended promoter-primer) is destroyed by RNase H (Figure 3). Schuster et al. teaches that this method cyclical and therefore can produce multiple copies (Column 4 lies 50-54). Therefore after the RNA is destroyed another primer can anneal to the nucleic acid sample.

With regard to Claims 145 and 148, Schuster et al. teaches a promoter primer which has at least 1 noncomplementary nucleotides (Figure 1 2nd step).

However, though Schuster et al. teaches that the primer can be RNA (Column 11, lines 47-51), Schuster et al. does not teach that the RNAse H is used to digest the substrate to remove the RNA segment of the primer.

Scheele et al. teaches a method for preparing a dsDNA from a ssDNA (abstract). Scheele et al. teaches a method of providing a first DNA strand, contacting with a primer, synthesizing in the presence of the primer that contains RNA and producing a copy of the DNA template (abstract). Scheele et al. teaches that RNA primers can be digested using RNA5e H (Column 6 lines 65-68).

Therefore it would be prima facie obvious to one of ordinary skill in the art to digest the RNA primer of Schuster et al. with the RNAse H which is already in the solution because Scheele et al. teaches that RNAse H digest RNA primers on a DNA/RNA hybrid strand. The ordinary artisan would be motivated to digest all RNA which is in the system in the double stranded form (e.g. DNAJRNA) in order to produce multiple copies of the nucleic acids of interest.[1]

The first obviousness rejection is respectfully traversed.

In response, Applicants respectfully make the following remarks. In Schuster et al., although there is reference to a "proto promoter," this is not the same element as a "proto-primer" or "promoter-primer," the latter two being terms which are used in the

[1] **The Examiner's Response to Arguments are found on pages 7-9 in the Office Communication.**

present Office Communication. Applicants believe that they are correct in this regard because Schuster et al. explicitly states in column 11, lines 59-61:

> Significantly, the 3' terminus of the molecule is blocked, and thus is **incapable of serving as a substrate for primer extension reactions**. [emphasis added]

Thus, the Schuster patent itself clearly notes that the nucleic acid hybridized to a target molecule is not a primer as understood by those of ordinary skill in the art. The characterizations on page 4 in the Office Communication that "Schuster et al., teaches an mRNA promoter (primer) which is used to extend and make ssRNA." and "the ssRNA (which is the extended promoter-primer)" are necessarily incorrect because the proto-promoter of Schuster is never itself extended.

The proto-promoter of Schuster serves two purposes. As shown in Figure 3, the proto-promoter first serves as a template for extension of the DNA analyte. Secondly, upon conversion of the promoter region into double-stranded form, the proto-promoter directs synthesis of RNA transcripts. This feature in Schuster's proto-promoter illustrates a critical difference between RNA and DNA synthesis because DNA polymerization always requires extension from a pre-existing primer whereas RNA transcription occurs de novo without a primer. During transcription, the Schuster "proto promoter" is **not** extended and it only serves to bind RNA polymerase molecules and to allow them to transcribe the target molecule as a template.

Furthermore, an RNA polymerase does not create an RNA/DNA hybrid except in a very transient sense. As an RNA polymerase transcribes a DNA template, the 3' end of the RNA is bound to the template but as the polymerase proceeds, the 5' continues to be removed from the template such that at the end of the transcription, the RNA is completely removed and the mRNA is free to be used as a template for protein synthesis. Numerous depictions of this aspect of the synthesis of mRNA can be seen in standard biology and biochemistry texts. This feature is also illustrated in Schuster's Figure 3, where in the step labeled "Transcribe RNA with RNA polymerase," the original

DNA analyte/template is not seen and the product is labeled as "ssRNA, antisense strand, complementary to the original DNA analyte." The depiction of a pair of dotted lines is the way that Schuster et al. choose to depict a ssRNA copy which is produced during a transcription reaction and is then released from its DNA template. Only later when an additional step takes place is the RNA transcript used as a template to make a cDNA copy that a true RNA/DNA hybrid is formed.

It is stated (page 4 in the Office Communication) that "the ssRNA (which is the extended promoter-primer) is destroyed by RNase H (Figure 3)". Again, this appears to be an improper use of the term "promoter-primer" as already discussed above, but in addition, this statement is also incorrect because ssRNA is never destroyed by RNase H. The specificity for RNase H is for a double-stranded nucleic acids where one strand is RNA and the other is DNA.

Comments on the specificity of RNase H were made in Applicants' earlier response in order to correct a mistaken idea that RNase H would digest unbound chimeric primers. Only in a later step in Schuster's Figure 3 are the RNA transcripts used to create complementary copies (cDNA) of the original analyte so that a substrate is formed which is suitable for RNase H digestion. This process is shown in three steps in Schuster et al. (Figure 3), where the first step is labeled "ANNEAL PRIMER A" which is a new primer (5'-A-3'), binding to the transcripts followed by the next step "SYNTHESIZE COMPLEMENTARY STRAND WITH REVERSE TRANSCRIPTASE" where Primer A is extended to form the product labeled "cDNA, SENSE OF ORIGINAL DNA ANALYTE" (i.e., this is a sense stand copy of the original analyte).

Applicants respectfully point out that Schuster's Figure 3 is incorrect to the extent that it retains the designation "ssRNA" for the bottom strand because it is only historically single-stranded but it is now part of a double-stranded DNA/RNA hybrid. Only then does this product in Schuster et al. serve as a substrate for RNase H digestion.

Schuster's product is not the original analyte from the first step of Figure 3 but rather a new copy that has been synthesized de novo. This can be seen where the original DNA molecule is labeled as "ssDNA analyte" at the top and the product in the bottom that re-enters the cycle is lableled "cDNA". On page 4 in the Office Communication, it is incorrect to characterize this cycle thusly as "Therefore after the RNA is destroyed another primer can anneal to the nucleic acid sample." [emphasis added.

There is no evidence in Schuster et al. that the original analyte sample can bind another primer since there never has been a step involving removal of the promoter oligonucleotide from the analyte. In Schuster's Figure 3, after the RNase H step, another primer (the proto-primer) binds to the new copy of the analyte, and not the analyte itself. This will then generate new transcripts and continue the cycle with a generation of a new round of products.

Applicants note that the claims have been amended to point out more particularly these differences by explicitly reciting that an extension of the primer takes place, that RNAse H action removes ribonucleotides from the extended primer and that the results of this digestion allows a new primer biriding/extension event.

As pointed out above, Schuster et al. provide no description for primer extension using ribonucleotides. Indeed, Schuster et al. provide no description for digesting an RNA segment of a primer. Moreover, Schuster et al. provide no description for a single site on a nucleic acid molecule that is used for more than one priming binding/extension event.

The deficiencies of Schuster are not cured by the addition of Scheele. As seen in Scheele's Figure 5, following extension of a ribonucleotide primer by DNA polymerase and treatment with RNAse H to remove the RNA portion of the extended primer, Scheele teaches away from using the exposed oligo C portion of the template for subsequent priming events by directing the user to eliminate this potential primer binding segment by the use of T4 polymerase (see final product in Scheele's Figure 5).

This step in Scheele renders the template unsuitable for use in another priming binding/extension event.

In view of the foregoing remarks and the above amendments to the claims, Applicants respectfully request reconsideration and withdrawal of the first obviousness rejection.

The Second Rejection Under 35 U.S.C. §103

Claim 122, 131-132, 144 and 147 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schuster etal. (US Patent 5,169,766 December 8, 1992) in view of Scheele (US Patent 5162209 November 10, 1992) as applied to claims 112-121, 123-130, 133-143, 145-146, 148 and further in view of Kacian et al. (US Patent 5554516 September 10, 1996). According to the Office Communication (pages 10-11):

> Schuster et al. and Scheele et al. teach a method of in vitro transcription. Schuster et al. teaches the use of primers which are chemically modified by blocking the 3' terminus using a 3' terminal nucleotide lacking a 3' hydroxyl group (Column 11 lines 60-64).
>
> Schuster et al. and Scheele et al., however, do not teach primers modified by heteroatoms comprised of nitrogen or sulfur and chemically modified primers comprised of nucleoside triphosphates or the use of copolymer primers.
>
> Kacian et al. teaches a method of amplifying a target nucleic acid sequence (Abstract). Kacian et al. teaches a method of incubating a promoter-primer and a target sequence in DNA priming and nucleic acid synthesizing conditions (ribonucleotide triphosphates and deoxyribonucleotide triphosphates) (nucleic acid precursors) for a period of time to many multiple copies of the target sequence (Column 10 lines 23-33).
>
> With regard to Claims 122, 131, and 132, Kacian et al. teaches that the 3' end of the promoter-primer may be modified (Column 7 line 6). Kacian et al. teaches that one modification can be the addition of a phosphorothioate (sulphur heteroatom) (Column 10 lines 22-33).
>
> With regard to Claims 144 and 147, Kacian et al. teaches the promoter-primer may be altered with ribonucleotides (Column 9, line 15). Therefore the promoter-primer can be RNA: DNA mixture. Kacian et al. teaches the promoter-primer can have both RNA and a DNA region (Column 9, line 15).

> Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Schuster et al. and Scheele et al. to use the modified ends and the copolymer primers as taught by Kacian et al. The ordinary artisan would have been motivated to modify the method of Schuster et al. and Scheele et al. to use the modified ends and the copolymer primers as taught by Kacian et al., because Kacian et al. teaches that primers can be blocked to inhibit extension from the 3' end of the primer such that only the nucleic acid sequence extending from the primer form the 3' end is amplified (column 6 lines 55-67 and column 7 lines 1-20). The ordinary artisan would be motivated to use copolymers as taught by Kacian et al. because Kacian et al. teaches that the promoter primer can include the addition of ribonucleotides or deoxynucleotides residues to effectively block the 3' end (Column 9 lines 10-25).

The second obviousness rejection is respectfully traversed.

In response, Applicants refer to and incorporate their remarks from the first obviousness rejection. In view of those remarks, it is respectfully submitted that the addition of Kacian et al. to Schuster et al. and Scheele does not cure the deficiencies in the latter two references.

Thus, the second obviousness rejection should and must be withdrawn because it is premised upon an improper characterization of the primary and secondary references which cannot be cured by including Kacian et al. as a tertiary reference.

Reconsideration and withdrawal of the second obviousness rejection is respectfully requested.

The Rejection For Double Patenting

Claims 91-101 stand provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 91-99 of copending U.S. Patent Application Serial No. 10/718391. According to the Office Communication (pages 13-14):

Although the conflicting claims are not identical, they are not patentably

distinct from each other because Claim 91-1 01 of the instant application describes the same method steps as Claim 91-99 of application 10/718391. Both applications are drawn to a method of producing copies of a specific nucleic acid by providing a nucleic acid sample, contacting it with unmodified nucleic acid precursors and modified RNA primers. Both applications use a catalyst. Both applications modify primers using hereoatoms comprising nitrogen or sulfur. Both applications claims are drawn to primers, which comprise about 1 to about 200 noncomplementary nucleotide or nucleotide analogs. The primers of the instant application are encompassed by the genus of generic primers claimed by 10/718391. The specification of 10/718391 defines a primer as DNA, RNA, or DNA:RNA, therefore the claims of 10/71 8391 are drawn to a method using a genus of primers which include the RNA primer and the DNA: RNA primer of the instant application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

In response, Applicants are submitting herewith accompanying this paper as Exhibit A their Terminal Disclaimer To Obviate A Provisional Double Patenting Rejection Over A Pending "Reference" Application [Form PTO/SB/25(12-08)].

In view of the submission of their Terminal Disclaimer (Exhibit A), Applicants respectfully request reconsideration and withdrawal of the double patenting rejection.

Early and favorable action is respectfully requested.

****

SUMMARY AND CONCLUSIONS

In the above claim listing for this application, claims 112, 114, 117, 120, 123, 125, 127, 130, 133, 135, 138, 139, 141, 143, 145, 146 and 148 have been amended; and claims 121-122 and 131-132 have been canceled. No claims have been added.

No claim fee is believed due for this paper because a fewer number of claims are being presented than the number of previously paid for claims. This paper is also accompanied by a Request For Extension Of Time (3 months) and authorization for the fee therefor. No other fee or fees are believed due in connection with this paper or the accompanying extension request. In the event that any other fee(s) is/are due in connection with this filing, however, the Patent and Trademark Office is hereby authorized to charge the amount of any such fee(s) to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone call would be helpful in the processing of this paper or this application, Applicants' undersigned attorney requests that he be contacted at the numbers below.

Respectfully submitted,

Ronald C. Fedus
Registration No. 32,567
Attorney for Applicants

ENZO LIFE SCIENCES, INC.
c/o ENZO BIOCHEM, INC.
527 Madison Avenue, 8th Floor
Previously Presented York, NY 10022-4304
Telephone: (212) 583-0100
Facsimile: (212) 583-0150

Enz-52(D2)(C)(D1)

Dean L. Engelhardt et al.
Serial No.: 10/713,183 (Filed November 14, 2003)
Exhibit A To Applicants' January 20, 2009 Amendment Under 37 C.F.R. §1.115
(In Reply To The July 24, 2008 Office Communication)

EXHIBIT A